Appl. No. 09/442,111
Amdt. dated April 12, 2007
Amendment under 37 CFR 1.116 Expedited Procedure
Examining Group 1652

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

- 1-52. (Cancelled)
- wherein the product saccharide is an oligosaccharide or glycolipid, the method comprising

 i) permeabilizing a microorganism or plant cell wherein the cell comprises:

 a) a heterologous accessory enzyme for forming a nucleotide sugar;

 and

 b) a heterologous glycosyltransferase;

 i) ii) contacting a the permeabilized microorganism or plant cell with an exogenous acceptor saccharide, wherein the cell comprises:

 a heterologous accessory enzyme for forming a nucleotide sugar;

 and

 b) a heterologous accessory enzyme for forming a nucleotide sugar;

 and

 b) a heterologous glycosyltransferase which catalyzes the transfer of a sugar from the nucleotide sugar to the acceptor saccharide to produce the product saccharide; and

 ii) iii) allowing formation of the nucleotide sugar and transfer of a sugar from the nucleotide sugar to the acceptor saccharide to form the product saccharide.
 - 54. (Cancelled)
- 55. (Previously presented) The method of claim 53, wherein the heterologous glycosyltransferase is endogenous to the cell and is produced by the cell at an elevated level compared to a wild-type cell.
- 56. (Original) The method of claim 53, wherein the product saccharide is produced at a concentration of at least about 1 mM.

Appl. No. 09/442,111
Amdt. dated April 12, 2007
Amendment under 37 CFR 1.116 Expedited Procedure
Examining Group 1652

57. (Currently amended) The method of claim 53, wherein the cell is permeabilized using 1% Xylene.

58-59. (Cancelled)

- 60. (Previously presented) The method of claim 53, wherein the heterologous accessory enzyme is one or more of:
- a GDP-mannose dehydratase, a GDP-4-keto-6-deoxy-D-mannose 3,5-epimcrase, and a GDP-4-keto-6-deoxy-L-glucose 4-reductase;
 - a UDP-galactose 4' epimerase;
 - a UDP-GalNAc 4' epimerase;
 - a CMP-sialic acid synthetase;
- a pyrophosphorylase selected from the group consisting of a UDP-Glc pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a GDP-mannose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase; a kinase selected from the group consisting of myokinase, pyruvate kinase, acetyl kinase, creatine kinase, UDP-Glc-dehydrogenase; and

pyruvate decarboxylase.

- 61. (Previously presented) The method of claim 53, wherein the heterologous accessory enzyme and the glycosyltransferase are expressed as a fusion protein.
- 62. (Original) The method of claim 61, wherein the fusion protein comprises a CMP-sialic acid synthetase activity and a sialyltransferase activity.
- 63. (Original) The method of claim 61, wherein the fusion protein comprises a galactosyltransferase activity and a UDP-Gal 4' epimerase activity.
- 64. (Original) The method of claim 61, wherein the fusion protein comprises a GalNAc transferase activity and a UDP-GlcNAc 4' epimerase activity.

Appl. No. 09/442,111
Amdt. dated April 12, 2007
Amendment under 37 CFR 1.116 Expedited Procedure
Examining Group 1652

- 65. (Original) The method of claim 53, wherein the nucleotide sugar is GDP-fucose and the glycosyltransferase is a fucosyltransferase.
- 66. (Original) The method of claim 53, wherein the cell forms the nucleotide sugar at an elevated level compared to a wild-type cell.
- 67. (Original) The method of claim 66, wherein the elevated level of nucleotide sugar results from a deficiency in the ability of the cell to incorporate the nucleotide sugar into a polysaccharide normally produced by the cell.
- 68. (Original) The method of claim 67, wherein the deficiency is due to a reduced level of a polysaccharide glycosyltransferase activity.
- 69. (Original) The method of claim 53, wherein the cell/nucleotide sugar are selected from the group consisting of:

Azotobacter vinelandii/GDP-Man;

Pseudomonas sp./UDP-Glc and GDP-Man;

Rhizobium sp./UDP-Glc, UDP-Gal, GDP-Man;

Erwinia sp./UDP-Gal, UDP-Glc;

Escherichia sp./UDP-GlcNAc, UDP-Gal, CMP-NeuAc, GDP-Fuc;

Klebsiella sp./UDP-Gal, UDP-GlcNAc, UDP-Glc, UDP-GlcNAc;

Hansenula jadinii/ GDP-Man, GDP-Fuc;

Candida famata/UDP-Glc, UDP-Gal, UDP-GlcNAc;

Saccharomyces cerevisiae/UDP-Glc, UDP-Gal, GDP-Man, GDP-GlcNAc; and

X. campesti/UDP-Glc, GDP-Man.

70. (Original) The method of claim 53, wherein the cell is Azotobacter vinelandil, the nucleotide sugar is GDP-mannose, the acceptor saccharide is lactose, the glycosyltransferase is mannosyl transferase, and the product saccharide is mannosyl lactose.

Appl. No. 09/442,111
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Examining Group 1652

- 71. (Currently amended) The method of claim 53, wherein the cell is *E. coli*, the nucleotide sugar is CMP-sialic acid, the acceptor saccharide is lactose, the glycosyltransferase is a sialyltransferase, the heterologous accessory enzyme is CMP-sialic acid synthetase, and the product saccharide is sialyllactose.
- 72. (Previously presented) The method of claim 53, wherein the glycosyltransferase consists essentially of a catalytic domain of the glycosyltransferase.
- 73. (Previously presented) The method of claim 53, further comprising the step of detecting the product saccharide.
- 74. (Previously presented) The method of claim 53, further comprising the step of isolating the product saccharide.